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## A century of bottom-up- and top-down-driven changes on a lake planktonic food web: A paleoecological and paleoisotopic study of Lake Annecy, France

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### Abstract

We reconstructed the changes in the planktonic food web of an oligotrophic subalpine lake over the past century, combining paleoecological methods and historical monitoring data. Analyses of organic matter  $\delta^{15}\text{N}$  from sediment cores show that nutrient enrichment started in the 1910s and intensified from the 1930s. Subsequent changes in sediment organic carbon content and accumulation rates, carotenoid pigment concentrations, organic matter  $\delta^{13}\text{C}$ , and cladoceran subfossil remains show that excess nutrient inputs triggered bottom-up-driven increases in lake primary production, as well as in *Daphnia* abundance. *Daphnia* size, however, started to decrease in the late 1930s, indicating an increasing size-selective predation from zooplanktivorous whitefish populations (*Coregonus lavaretus*) that had been fostered by stocking and increased food availability. Whitefish predation is likely to have indirectly facilitated the establishment of *Bosmina longirostris*. With decreasing nutrient concentrations, *Daphnia* abundance decreased, but their size declined, presumably due to an ongoing size-selective predation. At this same time, *Bosmina* abundance doubled as a likely result of released interspecific competition from smaller *Daphnia*.  $\delta^{15}\text{N}$  analyses on subfossil cladoceran remains revealed that these changes in cladoceran community structure were accompanied by major changes in the food web. In spite of successful measures to reduce nutrient inputs from the late 1960s and reduced primary production, anoxia still occurs every summer at the lake bottom. These patterns are the consequence of a still relatively high export of phytoplankton-derived organic matter to the sediment as a result of strong top-down effects on the planktonic food chain.

The lesson of more than 30 yr of worldwide efforts to restore lake water quality is that cutting the apparent external causes of lake degradation does not guarantee lake recovery. Many lakes worldwide have undergone eutrophication, beginning in the early or mid 20th century (Edmondson et al. 1956; Hutchinson 1969; Thomas 1969). Considerable efforts have been made since then to cut nutrient inputs, especially phosphorus, which was identified as the major cause of the degradation of lake water quality (Schindler 1974). However, even though phosphorus concentrations could be significantly reduced in many lakes (Jeppesen et al. 2005), some of them tend to respond slowly to reduced phosphorus loading, implying that the reduction-triggered improvement in ecological state is not as substantial as could be expected (Sondergaard et al. 2007). Recovery of lakes may also be confounded by concomitant environmental changes such as global warming, human-mediated disturbances, and management decisions (Anneville et al. 2002). Hence, even in cases where strategies to reduce nutrient concentrations resulted in substantial decrease in phytoplankton biomass, oligotrophication can be accompanied by unforeseen changes in communities that prevent recovery along a simple reciprocal pathway (Battarbee et al. 2005).

Unraveling the underlying processes driving lake trajectories might be achieved through a food-web approach. Yet, few lakes worldwide have actually been subjected to long-time biomonitoring surveys. Long-term biological data sets covering the pre- and postrestoration time periods are rarely available, hence limiting direct studies. However, such questions could be indirectly addressed by resorting to palaeolimnological approaches. Lake sediments contain considerable information about a range of past lake communities (e.g., diatoms, cladocerans, chironomids). However, to date such studies have been mostly restricted to certain components of the archive (Anderson et al. 2008). Only few studies (Leavitt et al. 1989; Räsänen et al. 1992; Anderson et al. 2008) have actually combined multiple biotic proxies in spite of a great potential for reconstructing past changes in the structure of the different biological compartments within a food-web perspective, i.e., relating structural changes in lake communities to functional changes of lake food web, as stressed by Jeppesen et al. (2001).

Such food web perspectives could be considerably improved by the addition of stable isotope analyses (SIA) to these classical paleoecological approaches. SIA have been extensively used to study contemporaneous trophic relationships in lakes worldwide over the last 25 yr (Kling et al. 1992). Besides, carbon and nitrogen SIA of sediment

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organic matter (OM) also belong to the classical toolbox of paleolimnologists. Changes in the  $\delta^{13}\text{C}$  values of OM along sediment cores have been used to track changes in lake pelagic primary production (Schelske and Hodell 1991), whereas changes in sediment  $\delta^{15}\text{N}_{\text{OM}}$  have been related to changes in nitrogen sources to the lake (Hobbs and Wolfe 2007) and nitrate utilization by primary producers (Teranes and Bernasconi 2000). SIA on sediment OM can therefore document past changes in C and N fluxes at the base of the food web. However, until now, studies of changes in C and N fluxes within the food webs using SIA of cladoceran subfossils are very scarce (Struck et al. 1998), although they could potentially fill a gap (Perga in press). Combining paleoecological and paleoisotopic techniques could provide a comprehensive approach of changes in lake pelagic food webs and improve our understanding of the processes driving lake trajectories as a result of perturbations and restoration efforts.

Our study focuses on Lake Annecy, a deep, large, and oligotrophic French subalpine lake. In the 19th century, the lake was considered as fish poor, with low yields of the dominant species (bleak [*Alburnus alburnus*], common dace [*Leuciscus* sp.], and perch [*Perca fluviatilis*]; Le Roux 1908). No obligate large zooplanktivorous fish species were present at this time (Poulet 1866). Whitefish (*Coregonus lavaretus*) was introduced in 1886 to enhance the fish productivity of Lake Annecy. Seven years later, fishermen reported whitefish catches (Le Roux 1908). Whitefish were stocked in the lake sporadically from 1900 to the 1930s and then annually every winter from 1936 to 1997. Ever since its introduction, whitefish have been the major fisheries resource in this lake. Since whitefish are essentially zooplanktivorous in subalpine lakes (Berg et al. 1994; Perga and Gerdeaux 2005), whitefish introduction is expected to trigger changes in the structure of the zooplankton prey communities that could eventually cascade down to phytoplankton, i.e., modify the top-down control on the pelagic food web (Carpenter et al. 2001). In addition, in the early 1940s, first symptoms of increasing lake trophic status were reported by local authorities. In the mid 1960s, Lake Annecy turned from oligotrophic to mesotrophic (Druart and Pelletier 1998). Measures against eutrophication were taken in 1967, when a sewage collector was built around the lakeshore to control anthropogenic nutrient inputs from the watershed. Such changes in the water nutrient concentrations are likely to drive bottom-up-controlled changes in the upper pelagic food chain (Carpenter et al. 2001).

The aim of this study is to reconstruct the potentially interacting bottom-up- and top-down-driven changes in the lake food web triggered by these perturbations, using paleoecological, paleoisotopic methods and historical monitoring data, to assess whether the apparent successful restoration of the water quality of Lake Annecy also triggered the recovery of its original planktonic food web.

## Methods

**Environmental setting**—Lake Annecy is a deep monomictic lake located in the French Alps at about 600 m

above sea level. It is 14.6 km long and 2 km wide, and its total area is 27.4 km<sup>2</sup> and its maximum depth 69 m. The lake water retention time is 4 yr. The lake is divided into two basins that lie in a tectonic depression that was reshaped by glaciers. The catchment area of 254 km<sup>2</sup> is drained by seven streams that rework marine marly formations (Jurassic to Low Miocene) and glaciolacustrine formations (Quaternary) and is essentially covered by forests (47%), rocky areas (22%), and meadows (15%) with a low proportion of agricultural areas (16%). First human effects on the lake watershed were identified as soon as 1700 B.P., following the Roman colonization (Noel et al. 2001). However, human activities around the lake shore have substantially intensified from the late 19th century with the population of the city of Annecy (the main city on the watershed) growing from 10,000 inhabitants in 1870 to 186,000 inhabitants in 2007 (Source: National Institute for Statistics and Economic Studies; [www.insee.fr](http://www.insee.fr)).

**Historical data**—The recent history of the lake was documented using discontinuous monitoring data (Syndicat Intercommunal du Lac d'Annecy—National Institute for Agronomical Research long-term database). Chemical, physical, and some biological data were sporadically collected between 1968 and 1980 and, since 1996, the lake has been subjected to a monthly or bimonthly monitoring survey. Only parameters for which collection, analytical methods, and sampling frequency were comparable between these two time periods were retained for this study. Phytoplankton countings that had been performed from net-collected samples during the earliest surveys and from depth-integrated water samples since 1996 cannot be used to compare the two time periods. Total phosphorus concentrations ( $P_{\text{tot}}$ ) were measured using the acid molybdate method (AFNOR) after digesting unfiltered samples with potassium peroxodisulfate. Presented  $P_{\text{tot}}$  concentrations are those measured during winter mixing in 1971, 1974–1980, and 1990–2006. Chlorophyll *a* (Chl *a*) concentrations in the water column were measured using spectrophotometry (Strickland and Parsons 1968) every month at 3 and 10 m since 1992. O<sub>2</sub> concentrations at the lake bottom were measured by the Winkler method (AFNOR) in 1974–1980 and 1996–2004. Zooplankton were collected every month with a 200- $\mu\text{m}$  mesh-size zooplankton net along 0–20-m vertical hauls during the 1968–1974 time period and since 1996.

**Coring and chronology**—In December 2006, two short cores (cores 06-01 and 06-03 with a total length of 30 and 40 cm, respectively) were recovered from the deepest part of the lake at 68-m water depth using a gravity corer. Both cores were sampled at 0.5-cm intervals and correlated using <sup>137</sup>Cs profiles. Radionuclide activities were measured by ultralow background gamma-spectrometry at the “Laboratoire Souterrain de Modane” (Reyss et al. 1995).

The most widely used method for dating recent lacustrine sediments is <sup>210</sup>Pb<sub>exc</sub> ( $T_{1/2}$  = 22.3 yr) method, which produces age–depth relations for the last 100 to 150 yr. Despite several improvements, this method based on constant <sup>210</sup>Pb and sediment fluxes and a static position

of radionuclides at a horizon level within the core after deposition does not always lead to precise ages and it has to be completed by others. The addition of artificial radionuclide ( $^{137}\text{Cs}$  and  $^{241}\text{Am}$ ) profiles allows an unambiguous dating of one or two events: the Tchernobyl accident in 1986 with a  $^{137}\text{Cs}$  peak and the maximum of atmospheric testing of weapons fallout in 1963 with high activities of both  $^{137}\text{Cs}$  and  $^{241}\text{Am}$  (Appleby 2000; Nomade et al. 2005). When a good agreement with the two methods is achieved and as far as the decay of  $^{210}\text{Pb}_{\text{exc}}$  with depth remains exponential, the  $^{210}\text{Pb}_{\text{exc}}$  method is assumed to be efficient and may allow the dating of sediments deposited at the end of the 19th century and during the 20th century.

**Analytical techniques**—Sediment chemistry: Percentage dry weight was calculated by weighting a known volume of wet core samples, drying, and reweighting. Dry weight accumulation rates (dry wt AR) were assessed from sedimentation rate and sediment dry weight per unit volume. Total element concentrations of carbon, nitrogen (TN), and sulfur (TS) were determined as weight percentages on freeze-dried samples from core 06-01 using an elemental analyzer (EuroEA, Eurovector). To measure total organic carbon (TOC), preweighted subsamples were acid-treated with 3% and 20% HCl to remove any carbonates present and then measured with the elemental analyzer. Ratios of TOC:TN (hereafter referred to as C:N) were calculated by weight. Organic carbon accumulation rate (OCAR) was estimated by multiplying dry wt AR by %TOC.

**Sedimentary pigment analyses**—Samples for pigment analyses (core 06-01) were freeze-dried, homogenized with mortar and pestle, and stored protected from light at  $-80^{\circ}\text{C}$ . Pigment analyses were conducted in the dark on weighted 200–300 mg of sediment. After adding 2 mL of ice-cold extraction solution (methanol +  $0.5 \text{ mol L}^{-1}$  ammonium acetate) samples were sonicated for 15 min in ice and vortexed. Extraction was continued overnight at  $-20^{\circ}\text{C}$  and samples were one more time sonicated and vortexed before centrifugation for 5 min at 3000 rotations per minute at  $4^{\circ}\text{C}$ . One milliliter of supernatant was transferred into vials for analyses by high-performance liquid chromatography. Samples were loaded on an autosampler (Waters 717plus Autosampler) equipped with a  $200\text{-}\mu\text{L}$  injection loop. The separation of pigments was performed on a Phenomenex Luna  $5\mu\text{ C18(2)}$  column ( $250 \times 4.60 \text{ mm}$ ) under a flow of three successive eluants (eluant A: 80:20 v:v methanol,  $0.5 \text{ mol L}^{-1}$  ammonium acetate; eluant B: 90% acetonitrile; eluant C: ethylacetate). Flow rate was  $1.0 \text{ mL min}^{-1}$  and absorbance was read at the maximum absorbance wavelengths of the targeted pigments (see below). Peak areas were converted to concentrations by the external standard calibration method using commercially available pigment standards (Dionex Canada) for Chl *a* (431 nm), pheophytin *a* (410 nm), zeaxanthin + lutein (448 nm; the close retention times for these two pairs of pigments did not allow reliable separation from each others), alloxanthin, and diatoxanthin + dinoxanthin (454 nm). Labile carotenoids (fucoxanthin) and chloro-

phylls (Chl *a*, *b*, and pheophytin *a*) were excluded from the subsequent data interpretation and statistical analyses to avoid problems associated with degradation (Leavitt and Findlay 1994). Fossil pigment abundances were presented as organic carbon-specific concentrations ( $\mu\text{g g}^{-1} \text{ OC}$ ).

**Cladoceran analyses**—Cladoceran remains were analyzed from core 06-03 according to Frey (1986). Wet sediment (1–4 g) was deflocculated in 100 mL of 10% KOH at  $85^{\circ}\text{C}$  for 30 min and passed through a  $31\text{-}\mu\text{m}$  sieve. The concentrated remains were then stained using safranin. As remains abundances were low, samples were entirely counted. Subfossil remains were identified using the determination keys of Frey (1986) and a standard microscope at 100–200X magnification. For *Bosmina*, most abundant and identifiable subfossil remains were head shields from the two *Bosmina* species historically identified in Lake Annecy (*Bosmina longirostris* and *Eubosmina longispina*; Balvay and Druart 1992). Identifiable *Daphnia* remains were postabdominal claws. The present *Daphnia* population in Lake Annecy is composed of *Daphnia hyalina* and *Daphnia galeata* and their hybrids *D. hyalina*  $\times$  *galeata*. Distinguishing *Daphnia* species from postabdominal claws is difficult and ehippia were much rarer than claws in Lake Annecy sediments and did not allow reliable countings. *Daphnia* remains were identified at the generic level (*Daphnia* sp.). Results were reported as number of head shields (for *Bosmina* species) or postabdominal claws (for *Daphnia* sp.) per gram of wet sediment. Errors associated with subfossil countings were estimated from six triplicate samples in which the average abundance of *Daphnia* sp. remains spanned from 7 to 180 individuals ( $\text{g sediment}^{-1}$ ) and that of *Bosmina* sp. remains from 19 to 255 individuals ( $\text{g sediment}^{-1}$ ). Average coefficients of variation over the six triplicates were  $< 0.3$  and  $< 0.2$  for countings of *Daphnia* and *Bosmina* remains, respectively.

The length of the *Daphnia* postabdominal claws was well correlated to individual size ( $n = 100$ ,  $r^2 = 0.83$ ,  $p < 0.001$ ) in contemporaneous samples. *Daphnia* sp. subfossil claws were hence measured to assess relative changes in the size structure of the *Daphnia* population.

**Stable isotope analyses**—Bulk sediment was treated with  $1 \text{ mol L}^{-1}$  HCl in a mortar to remove carbonates, thoroughly rinsed with distilled water, oven-dried at  $60^{\circ}\text{C}$ , and packed into tin cups. For Cladocerans, isotope analyses were performed only on *Bosmina* remains, as *Daphnia* remains were not abundant enough. Wet sediment from core 06-03 was stirred in demineralized water, retained on a  $31\text{-}\mu\text{m}$  filter cup, and thoroughly rinsed. *Bosmina* remains (head shields and carapaces) were visually checked for the absence of OM, manually picked under binocular to reach 0.3 mg of dry weight, and packed into tin cups. All isotope measures were performed at the SINLAB, New Brunswick, Canada, on a Finnigan Delta Plus mass spectrometer interfaced via a Conflo II to a NC2500 Elemental Analyzer.

Further analyses, however, suggested that the presence of carbonate coating on *Bosmina* remains might alter their



$\delta^{13}\text{C}$  values (Perga in press). Hence, rigorous measures of *Bosmina* remains  $\delta^{13}\text{C}$  would require prior acid treatment to remove carbonates. Unfortunately, there was not enough material left to rerun analyses. Results for *Bosmina*  $\delta^{13}\text{C}$  values are therefore not presented herein.

The relationships between the N isotope compositions of the exoskeletons and those of the organisms' whole body were investigated for two zooplankton taxa (*Bosmina* sp. and *Daphnia* sp.) from modern samples (Perga in press). The  $\delta^{15}\text{N}$  values of cladoceran exoskeletons vs. the whole body were shown to be strongly correlated but exoskeletons were strongly depleted in  $^{15}\text{N}$  by  $-7.9\%$  (standard deviation =  $0.5\%$ ).  $\delta^{15}\text{N}$  values of the *Bosmina* parent population from which the exoskeleton remains originated were hence assessed as

$$\delta^{15}\text{N}_{\text{Bosmina}} = \delta^{15}\text{N}_{\text{Bosmina remains}} + 7.9\% \quad (1)$$

Variations in *Bosmina*  $\delta^{15}\text{N}$  values over time can result from a change in trophic level or from a change in the isotopic composition of the primary producers (Cabana and Rasmussen 1996). Changes in the  $\delta^{15}\text{N}$  values of pelagic producers, or pelagic baseline, over time are reflected by changes in  $\delta^{15}\text{N}$  of sediment OM. Hence, the relative change in *Bosmina*  $\delta^{15}\text{N}$  that can be attributed to changes in its trophic position over time,  $\Delta\delta^{15}\text{N}$ , was estimated using

$$\Delta\delta^{15}\text{N} = \delta^{15}\text{N}_{\text{Bosmina}} - \delta^{15}\text{N}_{\text{OM}} \quad (2)$$

**Statistical analyses**—Chronological clustering (R-Package, Legendre and Vaudor 2000) was used to segment multivariate time series separately for cores 06-01 and 06-03. Data included in the chronological clustering were TOC,  $\delta^{15}\text{N}_{\text{OM}}$ ,  $\delta^{13}\text{C}_{\text{OM}}$ , and pigment contents for core 06-01; *Daphnia* claws abundance and average size; as well as abundances of *Bosmina* and *Eubosmina* head shields for core 06-03. Data were normalized using the Box-Cox Bartlett transformation in the VERNORM program (R-Package). Chronological clustering was computed using the CHRONO program (R-Package). Similarity matrices were first computed from the normalized data using the Gower similarity (Legendre and Vaudor 2000) in the SIMIL 3.0 program (R-Package). The nonhierarchical method of CHRONO uses a hierarchical proportional-link linkage algorithm whose connectedness level was set at 0.5. The constraint of temporal contiguity imposed to the clustering results means that only objects or object groups that are adjacent along the series may eventually cluster. The null hypothesis of that test is that the two groups being tested are an artifact and should be fused in a single group. The probability level  $\alpha$  was set at 5%. At each step of the agglomeration, a permutation test ( $n = 2000$ ) was performed to decide whether a fusion should be made between the two groups whose fusion was proposed by the agglomerative algorithm. CHRONO also includes a posteriori tests. First, each group in turn was expanded, assuming that the other groups do not exist and their objects are unclustered and available for clustering. Group expansion made it possible to determine whether the groups formed

were abruptly separated from one another (succession by jumps, or "saltation"), or if the transition among groups is smooth (gradual succession). Furthermore, a second series of a posteriori tests was used to look at the relationships among distant groups, to determine if some of them are similar (Legendre and Vaudor 2000). Univariate statistical analyses were performed using nonparametric tests (Kruskal-Wallis and Wilcoxon tests, Mann Kendall's tau) to avoid autocorrelation or homoscedasticity issues.

## Results

**Lake Annecy recent history**— $\text{P}_{\text{tot}}$  concentrations in Lake Annecy water column have been continuously decreasing for the last 30 yr, from  $16 \mu\text{g P L}^{-1}$  in 1971 (4 yr after the collector was built) down to  $\sim 6 \mu\text{g P L}^{-1}$  in the early 1990s.  $\text{P}_{\text{tot}}$  concentrations have been stable and low ( $< 6 \mu\text{g P L}^{-1}$ ) since then (Fig. 1A). Annual mean Chl *a* concentrations in the water column at 3 and 10 m have been also low and stable ( $< 4 \mu\text{g L}^{-1}$ ), with maximum concentrations at the spring peak below  $9 \mu\text{g L}^{-1}$ , since the mid 1990s (Fig. 1B). Such Chl *a* concentrations are typical for oligotrophic lakes, and consistent with the current  $\text{P}_{\text{tot}}$  concentrations since the early 1990s (Wetzel 2001).  $\text{O}_2$  concentrations show temporary lake anoxia at the bottom of the lake during summer for the whole time period, with complete  $\text{O}_2$  replenishment in winter as a result of complete water mixing (Fig. 1C). In spite of decreasing P concentrations, bottom lake anoxia during summer has persisted up to now (Fig. 1C). Annual mean *Daphnia* sp. abundance in the water column has significantly decreased between the 1968–1974 and the 1996–2006 time periods (Wilcoxon rank sum test,  $p = 0.04$ ), current average abundances being  $\sim$  three times lower than in the 1970s (Fig. 2A), consistent with what is expected for a reoligotrophicating lake. Annual mean *Bosmina* abundances did not show such strong changes between the two time periods (Fig. 2B, Wilcoxon rank sum test,  $p = 0.06$ ).

**Chronology**—For core 06-03, the  $^{137}\text{Cs}$  activity vs. depth profile showed a well-defined peak at 10-cm depth (Fig. 3A). The profile of  $^{241}\text{Am}$  with a similar trend confirmed for this peak the weapon fallout maximum in 1963. This age corresponded to an averaged sedimentation rate between 1963 and 2006 of  $2.3 \text{ mm yr}^{-1}$ . The small peak of  $^{137}\text{Cs}$  visible at 4.75-cm depth without any increase of  $^{241}\text{Am}$  activity was identified as the Tchernobyl fallout in 1986. This chronological marker implied a sedimentation rate of  $2.4 \text{ mm yr}^{-1}$  between 1986 and 2006 that was in good agreement with the averaged sedimentation rate between 1963 and 2006. The  $^{210}\text{Pb}_{\text{exc}}$  profile (i.e., total  $^{210}\text{Pb}$  minus  $^{210}\text{Pb}$  supported: equal by  $^{226}\text{Ra}$ ) for core 06-03 (Fig. 3B) followed an exponential relation with depth corresponding to a sedimentation rate of  $2.37 \text{ mm yr}^{-1}$ . The good agreement between sedimentation rates deduced from  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  profiles allowed the extrapolation of the regular sedimentation rate of  $2.37 \text{ mm yr}^{-1}$  giving 140 yr of sedimentation in core 06-03.

The  $^{137}\text{Cs}$  profiles of cores 06-01 and 06-03 were compared in Fig. 3C. The weapon fallout maximum of

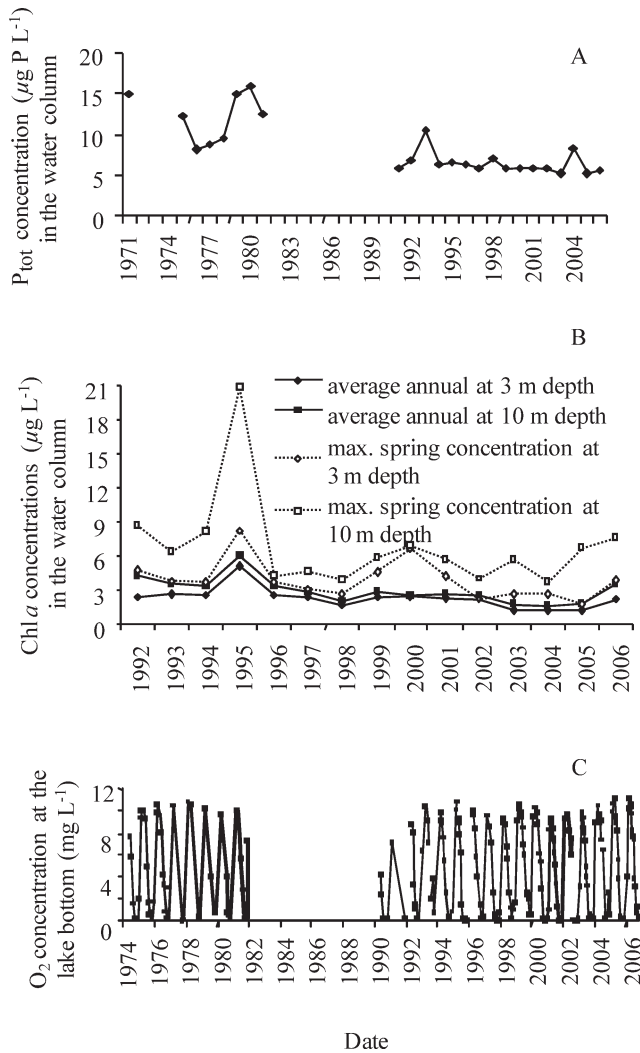


Fig. 1. Monitored changes in Lake Annecy chemical conditions: (A) Total phosphorus concentration in the water column during winter mixing in Lake Annecy in 1971–2006; (B) average annual chlorophyll *a* concentrations and values at the spring peak in Lake Annecy water column at 3 and 10 m in 1992–2006; and (C) monthly changes in O<sub>2</sub> concentration at the lake bottom in 1974–2006.

1963 was found at the same depth in the two cores and thus sedimentation rates for the two cores were assumed to be similar. Dry wt AR decreased slightly from 0.08–0.1 mg cm<sup>-2</sup> yr<sup>-1</sup> below 15-cm depth to 0.05 mg cm<sup>-2</sup> yr<sup>-1</sup> at the uppermost part of the core.

**OM and pigments (core 06-01)**—Six homogeneous time periods could be identified from CHRONO analyses with gradual transitions at the following dates: 1912–1914; 1937–1939; 1964–1966; 1980–1982; and 1989–1991 (Fig. 4A).

Vertical profiles were generally similar for TOC, OCAR, C:N, and the carotenoid pigments lutein + zeaxanthin and diatoxanthin + dinoxanthin (Fig. 4). Sediment TOC and OM  $\delta^{13}\text{C}$  values were negatively correlated ( $r^2 = 0.88$ ,  $p < 0.001$ ).

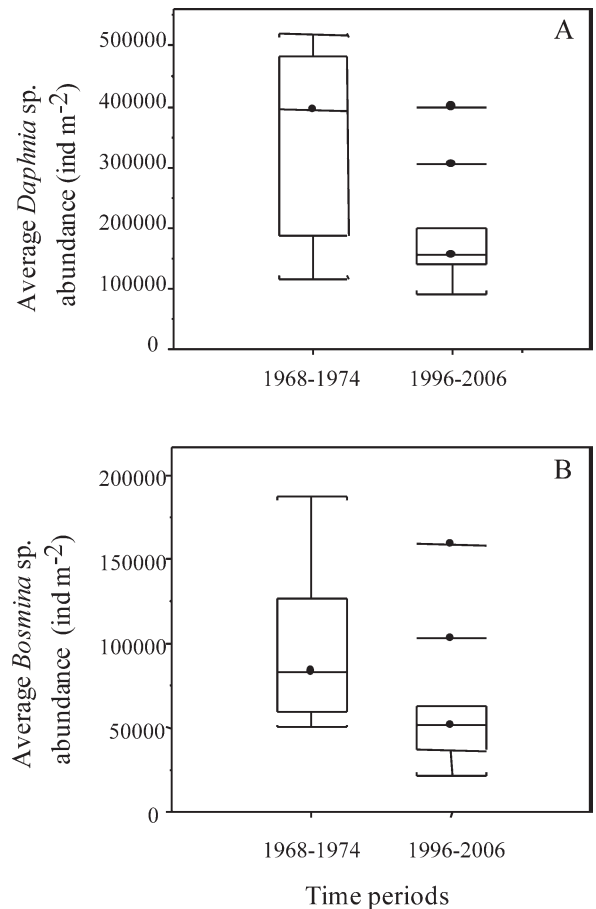


Fig. 2. Changes in (A) *Daphnia* sp. and (B) *Bosmina* sp. abundances in Lake Annecy between the 1966–1974 and 1996–2006 time periods.

Before 1912–1914, TOC, OCAR, and sediment contents in lutein + zeaxanthin (originating from chlorophytes and cyanobacteria) and diatoxanthin + dinoxanthin (originating from diatoms and dinoflagellates) were low, such as  $\delta^{15}\text{N}_{\text{OM}}$ , suggesting that at the end of the 19th century, nutrient inputs to the lake, and therefore primary production, were low. For this same time period,  $\delta^{13}\text{C}_{\text{OM}}$  exhibited the highest values of around  $-29\text{‰}$ , which might be consistent with substantial contribution of terrestrially derived OM. This is in contrast to low C:N ratios of around 8, which is typical for algal-derived OM. TS content was below detection limit.

$\delta^{15}\text{N}_{\text{OM}}$  started to increase after 1912–1914. Simultaneously, TOC, OCAR, and sediment contents in lutein + zeaxanthin and diatoxanthin + dinoxanthin slightly rose. At the same time,  $\delta^{13}\text{C}_{\text{OM}}$  values began to decrease. All these changes are consistent with a slow increased export of phytoplankton-derived,  $^{13}\text{C}$ -depleted, OM to the lake sediment.

From 1937–1939,  $\delta^{15}\text{N}_{\text{OM}}$ , TOC, OCAR, and pigment contents increased and  $\delta^{13}\text{C}_{\text{OM}}$  decreased substantially, showing the intensification of cultural eutrophication from the late 1930s to reach its maximum in 1964–1966. C:N ratio increased slightly as well. Sediment S could also be detected from this time period.

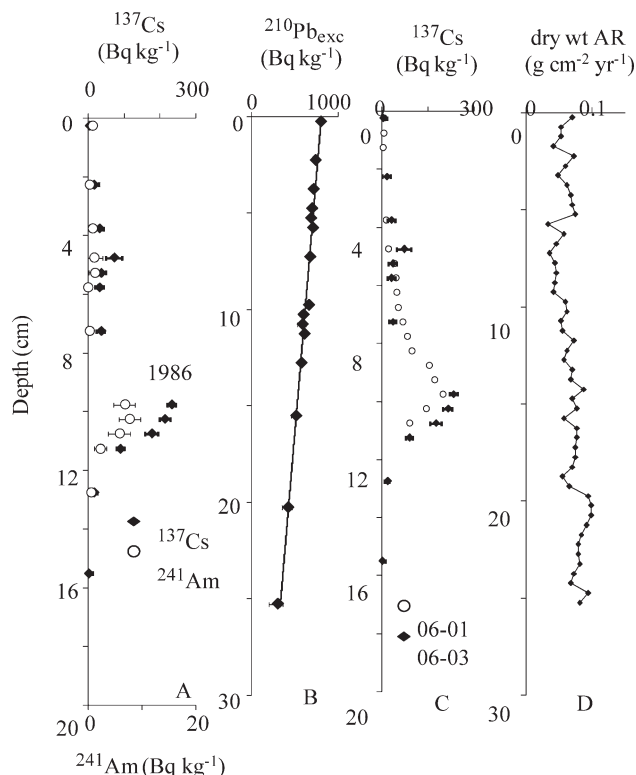


Fig. 3. Vertical profiles of (A)  $^{137}\text{Cs}$  and  $^{241}\text{Am}$ ; and (B)  $^{210}\text{Pb}_{\text{exc}}$  in core 06-03; (C)  $^{137}\text{Cs}$  in core 06-01 and 06-03; and (D) dry wt AR in core 06-01.

Between 1966 and the early 1980s, values for all analyzed proxies remained relatively stable, which suggested that eutrophication stopped right after the collector was built around the lake. Thereafter,  $\delta^{15}\text{N}_{\text{OM}}$  had been decreasing over the last 25 yr. TOC, OCAR, and  $\delta^{13}\text{C}_{\text{OM}}$  remained at the same values as they were during maximum eutrophication, hence implying that the contribution of phytoplankton-derived OM to the sediment was still high. The two last breaks identified by CHRONO corresponded to a time period during which sediment contents in lutein + zeaxanthin and diatoxanthin + dinoxanthin decreased briefly in the 1980s and increased again to reach values to similar to those of maximum eutrophication from the late 1980s. Allo-xanthin as well as S contents in the sediment, which were at low values so far, increased steadily in the last 15 yr.

*Cladocerans (core 06-03)*—Five homogeneous time periods were identified by CHRONO analyses (Fig. 5). A posteriori tests indicated an abrupt transition in 1910–1912 and smooth transitions in 1935–1937; 1950–1952; and 1967–1969. Hence, transitions in the early 1910s, mid-late 1930s, and late 1960s, which corresponded respectively to the beginning of early and slow eutrophication, the intensification of cultural eutrophication, and the date at which restoration measures were taken, were common in both time series of the OM and pigments (core 06-01) and cladocerans (core 06-03) descriptors (Fig. 5).

Vertical profiles of *Bosmina* and *Daphnia* sp. abundances were not significantly correlated (Fig. 5). Before 1910, the abundances of *Daphnia* sp. remains were relatively low but

variable and the average size of the claws was large, suggesting low to moderate abundances of large *Daphnia* sp. between 1860 and 1910. For the genus *Bosmina*, only remains from *E. longispina* could be identified in low abundances. CHRONO did not detect any significant rupture in the cladoceran community structure right after first whitefish introduction in 1886.

During the early eutrophication period between 1910 and the mid 1930s, *Daphnia* sp. abundance decreased to very low levels (Kruskal–Wallis test,  $p = 0.01$ ), with no significant change in their size (Kruskal–Wallis test,  $p = 0.22$ ), while *E. longirostris* became slightly more abundant (Kruskal–Wallis test,  $p = 0.02$ ). For this time period,  $\delta^{15}\text{N}$  of *Bosmina* remains was low, as for  $\Delta\delta^{15}\text{N}$ .

From the 1940s on, *Daphnia* abundance increased, following the intensification of cultural eutrophication. Average *Daphnia* sp. sizes, however, started decreasing simultaneously. The abundance of *E. longispina* did not change significantly but a new species, *B. longirostris*, appeared at low abundance in the mid 1940s.  $\delta^{15}\text{N}_{\text{Bosmina}}$  and  $\Delta\delta^{15}\text{N}$  increased simultaneously. The increase of *Daphnia* sp. was even more substantial after 1950, reaching its maximum at the maximum eutrophication in the late 1960s while the size of *Daphnia* sp. claws kept on shrinking over the same time period. While the total abundance of *Bosmina* (*E. longispina* + *B. longirostris*) did not exhibit strong changes during the whole eutrophication period, the contribution of *B. longirostris* to the total population increased, from less than 10% in 1950 up to 40% in 1967.  $\delta^{15}\text{N}_{\text{Bosmina}}$  remains and  $\Delta\delta^{15}\text{N}$  also increased steadily along with the proportion of *B. longirostris*.

From the late 1960s, changes in cladoceran remains abundances and cladoceran historical data can be directly compared. Although *Daphnia* sp. have always been dominant over the genus *Bosmina* in the water column, *Bosmina* remains are more numerous in the sediment, as a result of the well-known better preservation of *Bosmina* than *Daphnia* sp. remains (Frey 1986; Kattel et al. 2007). However, relative changes in cladoceran abundances are consistent between paleolimnological and historical monitoring data. *Daphnia* sp. abundance stopped increasing, and even decreased after the late 1970s, consistent with results from historical monitoring data. *Daphnia* sp. size, however, kept on decreasing and in the uppermost part of the core, *Daphnia* postabdominal claws were 33% smaller compared with the pre-eutrophication period (Mann Kendall's tau 1939–2006 = 0.669;  $n = 31$ , two-sided  $p < 0.001$ ). In contrast, *E. longispina* and *B. longirostris* abundances peaked steadily in the late 1960s and stabilized at these values since then consistently with results from historical monitoring data. Within the *Bosmina* population, the proportions of *E. longispina* and *B. longirostris* remained at constant ratio of ca. 1:1 during this time period. Similarly,  $\delta^{15}\text{N}_{\text{Bosmina}}$  and  $\Delta\delta^{15}\text{N}$  kept the same high values they reached during maximum eutrophication.

## Discussion

Historical data available for Lake Annecy are discontinuous but provide information for the period following



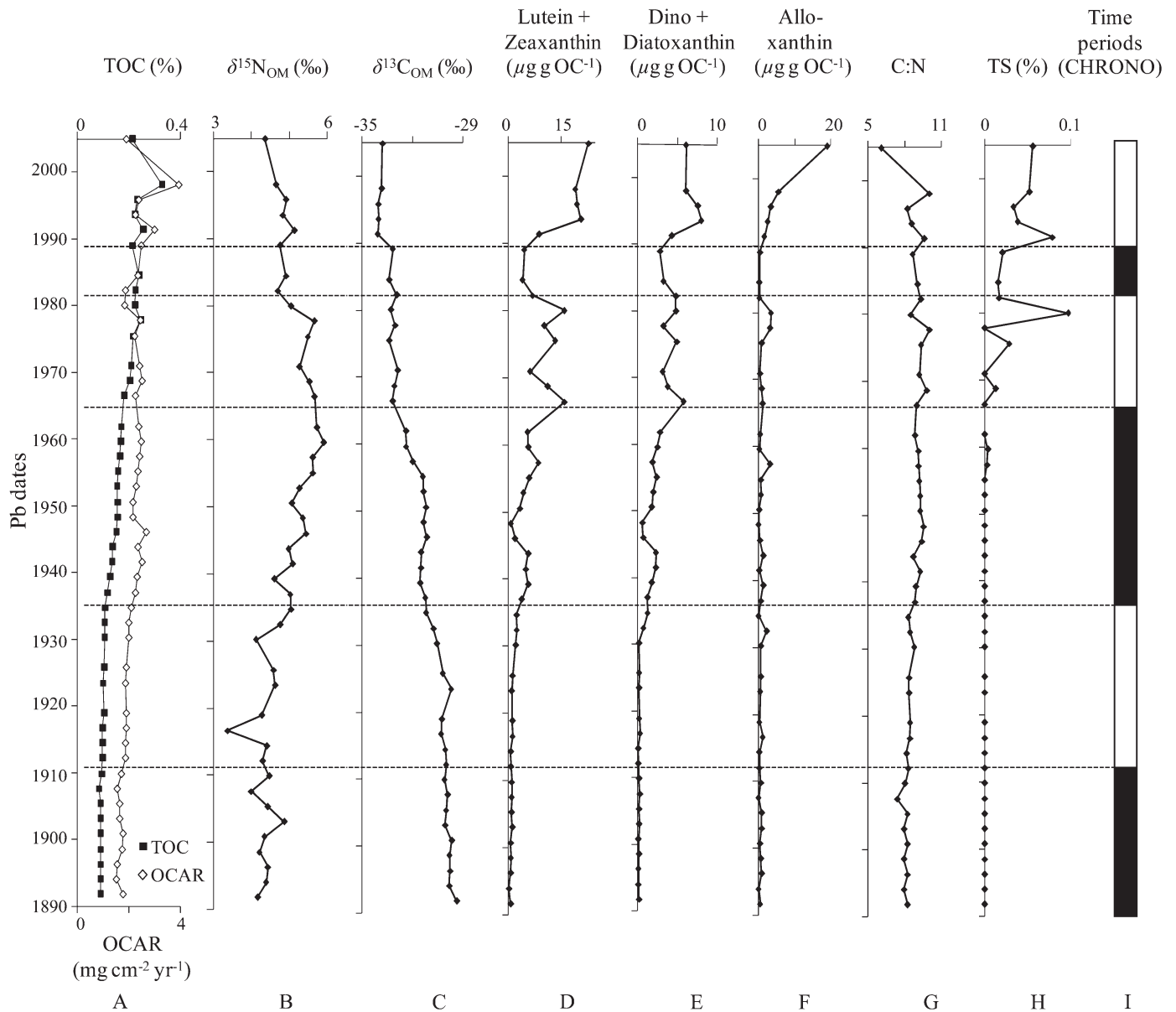


Fig. 4. Profiles of (A) TOC and OCAR; (B) OM  $\delta^{15}\text{N}$ ; and (C) OM  $\delta^{13}\text{C}$ ; concentrations in (D) lutein + zeaxanthin; (E) dinoxanthin + diatoxanthin; (F) alloxanthin; (G) C:N; and (H) TS; and (I) time periods identified by chronological clustering on core 06-01.

the collector construction in 1967. However,  $P_{\text{tot}}$  concentrations in 1971, i.e., 4 yr after the collector was built, were  $< 20 \mu\text{g P L}^{-1}$ , attesting that Lake Annecy was oligomesotrophic at its maximum eutrophication time.  $P_{\text{tot}}$  concentrations have been cut by three between the mid 1970s and the early 1990s, which attests to the efficiency of the restoration measures to reduce nutrient inputs to the lake. *Daphnia* sp. abundance in the water column has also been substantially reduced between the 1970s and the current time period.  $P_{\text{tot}}$ , Chl *a*, and zooplankton data all together suggest that restoration measures have been efficient and that Lake Annecy has reached, since at least the early 1990s, a stable oligotrophic status, according to criteria of Wetzel (2001). Surprisingly, despite apparent recovery of the lake water column and current low primary production,

transient summer anoxia is still persistent at the lake bottom. This suggests that the recovery of Lake Annecy does not occur along a simple reciprocal pathway and involves complex processes that could be documented through a paleoecological approach.

**History of eutrophication**—Before the early 20th century, low TOC, OCAR, cladoceran abundances, and pigments in the sediment were consistent with an oligotrophic status of Lake Annecy (Fig. 6A,B). C:N ratios were relatively low, around 8, which is a value more typical of a substantial contribution of algal-derived OM to the sediment (Meyers 2003). However, at low TOC values ( $< 1\%$ ), sedimentary C:N ratios can give misleading indications of bulk OM origin (Meyers 2003). Hence, at least in the lowermost

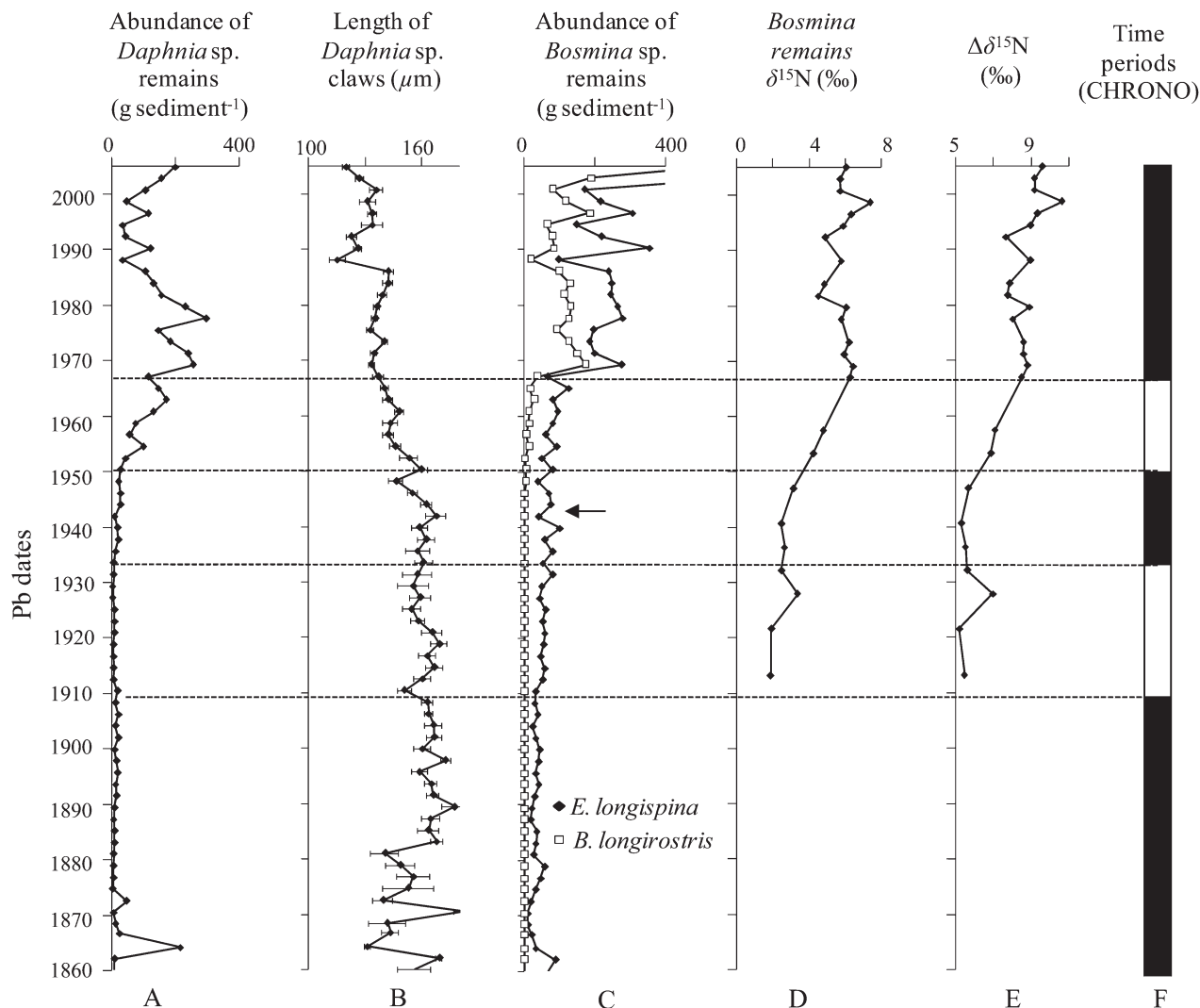


Fig. 5. Profiles of (A) abundance and (B) average length of *Daphnia* claws; (C) abundance of *E. longispina* and *B. longirostris* head shields; (D)  $\delta^{15}\text{N}$  of *Bosmina* remains; (E)  $\Delta\delta^{15}\text{N}$ ; and (F) time periods identified by chronological clustering on core 06-03. The arrow points the date at which the first remains of *B. longirostris* could be observed.

sections of the core, corresponding to the ultraoligotrophic state, C:N ratios might overestimate the contribution of algal-derived OM to the sediment, especially if inorganic N bound to minerals represented a significant proportion of N in the lowermost part of the core (Enters et al. 2006).  $\delta^{13}\text{C}_{\text{OM}}$  values were close to  $-28\text{‰}$ , a value that is typical of terrestrial-derived C. Previous paleolimnological studies in this lake confirmed, using Rock Eval pyrolysis, that organic carbon (OC) derived from soil and forest floor was three to four times more important to the sediment TOC than algal-derived OC before the beginning of the 20th century (Noel et al. 2001).

OM  $\delta^{15}\text{N}$  values have been used to track inputs of  $^{15}\text{N}$  heavy nitrogen from farm runoff and human sewage to a lake (Teranes and Bernasconi 2000), although the interpretation of this proxy might be hampered by other concurrent processes such as denitrification in bottom waters or changes in specific compositions of the phytoplankton (Talbot 2001; Meyers 2003). In this lake,

however, in the early 20th century, the bottom waters were oxic (see below); therefore changes in  $\delta^{15}\text{N}_{\text{OM}}$  cannot be attributed to denitrification processes. Changes in phytoplankton species composition during eutrophication might result in changes in  $\delta^{15}\text{N}_{\text{OM}}$  as N-fixing cyanobacteria developed in the water column, but such changes, contrary to our observations, should result in decreasing  $\delta^{15}\text{N}$  values for sediment OM (Talbot 2001; Meyers 2003). For such reasons,  $\delta^{15}\text{N}_{\text{OM}}$  efficiently tracked increasing anthropogenic nutrient inputs in Lake Annecy. Although the degradation of the quality of the lake water and decreased water transparency were reported for the first time in the early 1940s,  $\delta^{15}\text{N}_{\text{OM}}$ , TOC, OCAR, and pigment concentrations tracked the early eutrophication as soon as the 1910s, which corresponds to the beginning of the expansion of human population around the lake that followed the construction of a railway. Simultaneous increases in TOC, OCAR, and pigment contents, and decreasing OM  $\delta^{13}\text{C}$  in the sediment attested that higher nutrient concentrations in

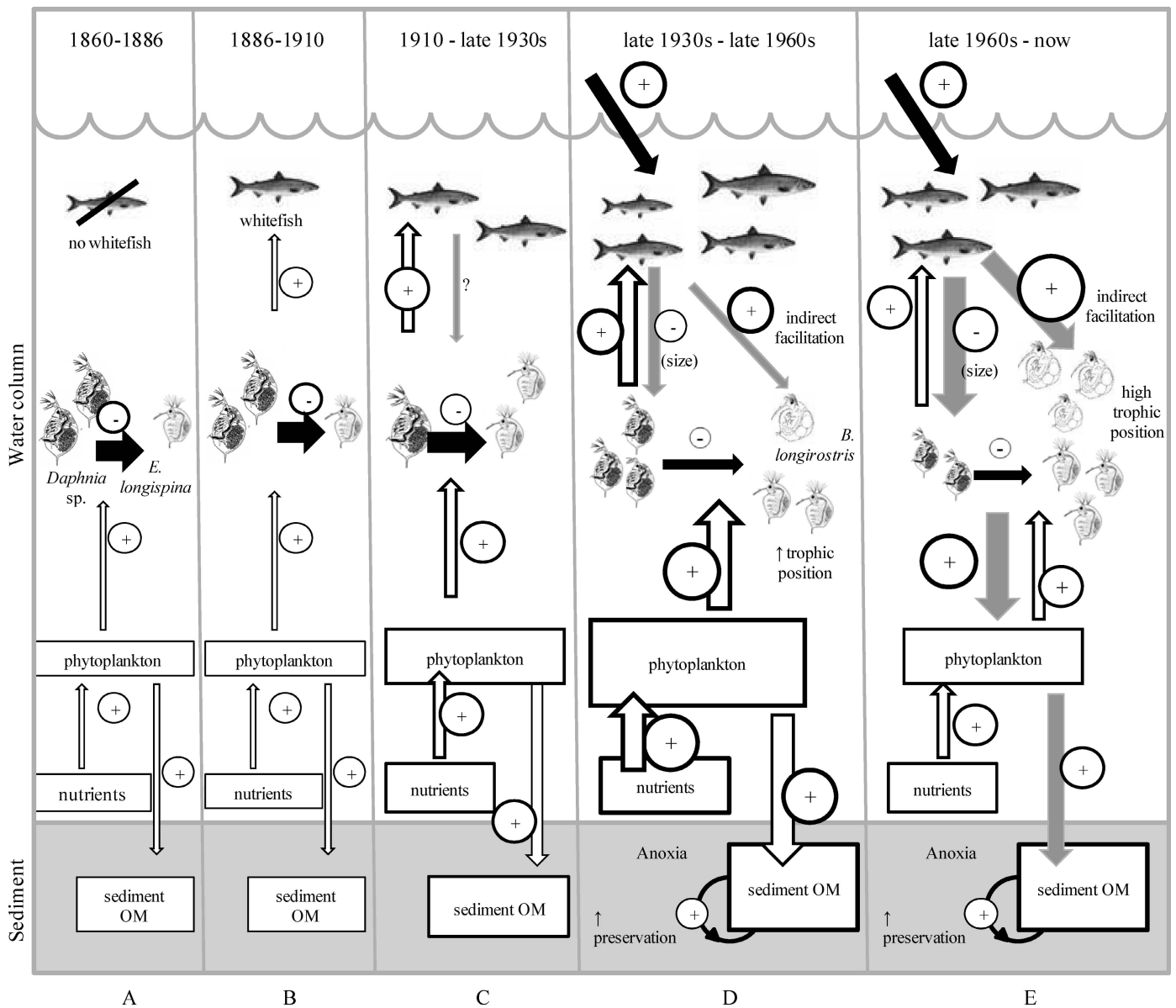


Fig. 6. Conceptual model of the interacting effects of whitefish introduction and changing nutrient levels on Lake Annecy planktonic food web and sedimentary processes for (A–E) the five identified time periods over the last 150 yr. Box size and line thickness increase with the relative biomass of the considered compartment (nutrients, phytoplankton, sediment OM). Open arrows refer to bottom-up controls, gray arrows to top-down controls, and black arrows to other kinds of effects such as competition or fish stocking. Arrow thickness and circle size increase with the relative strength of the considered effect.

the water column immediately resulted in an increased primary production from the major algae taxa, except for cryptophytes, and hence an increased export of  $^{13}\text{C}$ -depleted phytoplankton-derived OM to the sediment (Fig. 6C). From the mid-1930s, eutrophication intensified, likely as a result of industry and tourism expansion around the lake (Fig. 6D).

Sedimentary sulfur is derived from sulfate reduction, which depends on the development of anoxic conditions at the bottom of the lake, at least transiently during the season (Holmer and Storkholm 2001). Sediment sulfur content thus can provide information about redox condi-

tions of the sediment (Olsson et al. 1997) and is used as a proxy for the development of anoxia. No sulfur could be detected in the lowermost part of the core, indicating oxic conditions at the lake bottom before eutrophication. Sulfur contents increased once eutrophication intensified in the 1950s, suggesting that substantial anoxia has developed in the sediment as a result of increased input of pelagic OM and subsequent higher respiration rates in the bottom waters (Fig. 6D). Such results were confirmed from a recent study from chironomid subfossil remains that showed a clear transition from species typical of oxic conditions (*Microsetra* sp.) to species tolerant to anoxia

(*Sectrocladius* sp.), according to criteria of Quinlan and Smol (2001) as soon as the 1930s (L. Millet and V. Verneaux unpubl.).

*Changes in Cladocera community structure as a result of whitefish introduction and eutrophication*—Cladoceran community structure showed major changes for the same transition periods, i.e., early eutrophication in 1910s and intensification of cultural eutrophication in 1930s. However, responses of *Bosmina* and *Daphnia* abundances are complex and cannot be explained solely from bottom-up factors, but rather by an interaction between changing nutrient concentrations and top-down control by zooplanktivory.

Whitefish feed essentially on *Daphnia* for their whole life and select the largest individuals within the prey population (Berg et al. 1994; Perga and Gerdeaux 2005). Hence, strong effects of whitefish introduction on Cladocera, and especially *Daphnia* sp., community composition, and size structure could be expected (Vanni 1987; Berg et al. 1994). However, CHRONO did not detect any discontinuity in the cladoceran time series right after whitefish were introduced for the first time, suggesting that whitefish introduction did not instantly affect the cladoceran community structure (Fig. 6A,B). Such temporal delay between whitefish introduction and detectable effect on cladoceran structure has similarly been observed in Lake Pyhäjärvi, Finland (Räsänen et al. 1992). Planktivorous fish biomass is generally related to primary production (Carpenter et al. 2001). Consistently, whitefish populations were shown to be bottom-up controlled in oligotrophic lakes (Muller et al. 2007). Hence, as long as Lake Annecy was oligotrophic, the very low primary production could not sustain the establishment of an important whitefish population. In addition, whitefish tend to shift to benthic prey when *Daphnia* sp. food resource is limiting (Berg et al. 1994). It is then very likely that early populations of introduced whitefish did not exert a strong predation on *Daphnia* sp. Hence, at low nutrient concentrations and low levels of planktivory, *Daphnia* sp. were, as expected, large. *Daphnia* sp. and *Bosmina* sp. usually compete for food (DeMott and Kerfoot 1982). Grazing rates and the size spectrum of ingested particles are strongly dependent on the herbivore individual size ("size-efficiency hypothesis," Brooks and Dodson 1965; DeMott and Kerfoot 1982). Large-bodied zooplankton have greater per capita feeding rates and broader diets than smaller animals (Peters and Downing 1984). Hence, large *Daphnia* sp. were more efficient competitors than the smaller species *E. longispina* and could prevent the development of an abundant *E. longispina* population (Brooks and Dodson 1965; DeMott and Kerfoot 1982). This pattern remained apparently stable as long as the lake was oligotrophic, independently from the presence of whitefish (Fig. 6A,B).

The slight decrease in *Daphnia* sp. abundance during the early eutrophication period starting in the 1910s might result from an increase in predation by more abundant vertebrate planktivores, although, contrary to what was observed for this time period, such planktivory, which is usually size-selective, would be expected to come along with

reduced size of the prey species (Brooks and Dodson 1965; Vanni 1987). It has, however, to be considered that average size of *Daphnia* sp. over the 1910–1930s time period was measured on a relatively low number of individuals because of the scarcity of *Daphnia* sp. remains in this core section. The slight increase observed for *E. longispina* abundances after 1910 could result from a bottom-up effect of the higher primary production or a top-down consequence of a slightly released competition from *Daphnia* sp. (Fig. 6C).

In oligotrophic lakes, whitefish recruitment has been shown to be food limited (Gerdeaux and Anneville 2006; Muller et al. 2007). With intensified eutrophication from the mid 1930s, higher primary production could sustain a higher abundance of *Daphnia* sp. (consistent with what was previously observed in Lake Constance, Germany; Straile and Geller 1998), and therefore a more abundant food resource for whitefish. At moderate lake trophic status, whitefish biomasses increase because of better growth and survival of whitefish larvae and hence better recruitment (Gerdeaux and Anneville 2006; Muller et al. 2007). A higher biomass of whitefish is expected to exert an increasing size-selective predation pressure on the zooplankton prey species (Carpenter et al. 2001), resulting in a reduced size of the prey species (Mills and Schiavone 1982; Salo et al. 1989) (Fig. 6D).

Virtually all fish species present in the lake with at least one zooplanktivorous stage, such as perch (Post and Kitchell 1997), might benefit from a higher primary production and higher zooplankton abundance. A recent meta-analysis, however, showed that for European subalpine lakes, whitefish stocks have maximum values in oligomesotrophic lakes, i.e., the nutrient status Lake Annecy reached during its maximum eutrophication, whereas stocks of other fish species, such as perch, show significant increases only for mesoeutrophic nutrient status in subalpine lakes (Gerdeaux and Anneville 2006). Hence, it is very likely that whitefish, which have been additionally supported by annual fish stocking starting in 1936, was the main fish species responsible for such increase in size-selective zooplanktivory during the eutrophication phase.

Total *Bosmina* sp. abundance did not change significantly over these levels of eutrophication, but significant changes in the community structure occurred, with the appearance of *B. longirostris* in the late 1940s. The species transition between *E. longispina* and *B. longirostris* has been interpreted as a result of eutrophication (Deevey 1942; Boucherle and Züllig 1983). Such transition was actually postulated to result from an indirect predation-mediated effect rather than from a direct effect of increased nutrient inputs on the genus *Bosmina* (Brooks 1969; Kerfoot 1974). Our results support this hypothesis, because the appearance of *B. longirostris* coincided with the beginning of *Daphnia* sp. size reduction in the late 1940s. By reducing *Daphnia* size, whitefish-selective predation could indirectly reduce their competitive interaction with *Bosmina* sp. and allow the establishment of the new *Bosmina* species. This interpretation is consistent with indirect facilitation processes previously tested in mesocosms (Vanni 1987).

At the same time (ca. late 1940s),  $\delta^{15}\text{N}_{\text{Bosmina}}$  started to increase as well. Such an increase in  $\delta^{15}\text{N}_{\text{Bosmina}}$  cannot be



entirely attributed to diagenetic effects on the isotope composition of *Bosmina* sp. remains, as discussed in Perga (in press), but rather to an increasing trophic position for the whole *Bosmina* + *Eubosmina* group, which is supported by changes in  $\Delta\delta^{15}\text{N}$ . Such interpretations, however, hold as long as changes in  $\delta^{15}\text{N}_{\text{OM}}$  consistently reflect those in  $\delta^{15}\text{N}$  of pelagic primary producers, and by extension, those in the  $\delta^{15}\text{N}$  of nitrogen sources to primary producers. Independent processes, such as diagenesis and denitrification, might affect these relationships (Talbot 2001). Changes in  $\delta^{15}\text{N}_{\text{OM}}$  during eutrophication are very likely to reflect increased inputs from human waste and agricultural runoff. In addition, decreased  $\delta^{15}\text{N}_{\text{OM}}$  after the well-documented decrease in  $\text{P}_{\text{tot}}$  concentrations during the late 1960s attests to decreased nutrient inputs from the watershed into the lake. For such reasons, changes in  $\delta^{15}\text{N}_{\text{OM}}$  might indicate changes in primary producer  $\delta^{15}\text{N}$  values over time, as a result of changing N isotope composition of the N sources for primary producers, and hence might be an adequate pelagic baseline. A better estimate for this pelagic baseline might be reached using compound-specific N stable isotope compositions focused on phytoplankton-derived compounds, but these methods are still not readily available (Meyers 2003). Increased values for  $\delta^{15}\text{N}_{\text{Bosmina}}$  remains and  $\Delta\delta^{15}\text{N}$  from the late 1940s to the late 1960s are hence very likely the result of an increased trophic position for the whole *Bosmina*–*Eubosmina* group. *Bosmina* are able to switch from phytoplankton to heterotrophic flagellates depending on food conditions and interspecific competition (DeMott and Kerfoot 1982). Although there are so far no similar studies performed on freshwaters, marine zooplankton  $\delta^{15}\text{N}$  values were shown to increase with their respective reliance on protists vs. algae, i.e., their trophic position (El-Sabaawi et al. 2009). One interpretation could be that reduced competition due to smaller *Daphnia* sp. individuals and more abundant food resources might have allowed *Bosmina* sp. access to flagellate preys, as already observed in mesocosm experiments (Kerfoot 1987), and hence to a higher trophic position. However, our results do not allow us to determine whether only the newly appeared species *B. longirostris* or both *Bosmina* species occupied a higher trophic position.

*Changes in Cladocera community structure during reoligotrophication*— $\delta^{15}\text{N}$  values of sediment OM and the water column  $\text{P}_{\text{tot}}$  concentrations decreased soon after the collector was built, and the current Chl *a* concentrations and primary production of the lake are consistent with an oligotrophic status for Lake Annecy (Wetzel 2001). Altogether, these historical and paleolimnological data attest to the efficiency of the collector to control nutrient inputs from the watershed and of the subsequent reduction of the lake primary production. As expected under bottom-up control, both paleolimnological and historical data show a decrease of *Daphnia* sp. abundance during reoligotrophication as a result of decreasing food resource. In contrast, the trends observed during eutrophication for *Daphnia* sp. size and *Bosmina* sp. abundances, species composition, and trophic position did not reverse during

reoligotrophication. This suggests that these characteristics were not nutrient but rather top-down controlled. The observed trend in *Daphnia* sp. size suggests increasing predation pressure during reoligotrophication, whereas, at first sight, the opposite might be expected: With decreasing nutrient concentration, whitefish biomass and consequently predation pressure should decrease (Carpenter et al. 2001). However, until very recently (1997), whitefish have been annually stocked into the lake. Already at early stages, whitefish larvae select *Daphnia* sp. as preferred prey (Perga and Gerdeaux 2005). Our results therefore suggest that whitefish stocking contributed to maintaining a continuous strong predation pressure on a *Daphnia* sp. population whose abundance was already decreasing, and consequently triggered the observed continuous reduction in *Daphnia* sp. size even during reoligotrophication. Smaller *Daphnia* sp. were less and less competitive toward *Bosmina* and *Eubosmina*, whose abundances and trophic position stabilized at high values (Fig. 6E).

*Further top-down effects during reoligotrophication*—In spite of decreasing nutrient concentrations and low primary production in Lake Annecy, OCAR and TOC in the uppermost sediments stabilized at high values while  $\delta^{13}\text{C}_{\text{OM}}$  remained low during the whole reoligotrophication period. Pigment concentrations showed transient changes in the 1980s that could result from changes in phytoplankton community structure but remained generally at high values. In addition, anoxia still appears temporarily during summer at the lake bottom. These results suggest an ongoing high contribution of phytoplankton-derived OM to the sediment, despite decreasing primary production. Such pattern might be the consequence of top-down effects on phytoplankton-derived OM export to the sediment. Increasing whitefish predation pressure during eutrophication and reoligotrophication deeply altered the cladoceran community and size structure, triggering a switch from a community dominated by large-bodied grazers (i.e., large *Daphnia*) to a community dominated by small-bodied grazers (small *Daphnia*, *Bosmina*, and *Eubosmina*), with consequently lower per capita feeding rates and narrower size spectrum of edible particles (Peters and Downing 1984). During reoligotrophication, although primary production has been decreasing, a relatively more important fraction of this primary production could then not be grazed by the herbivorous zooplankton and could consequently be exported to the sediment. This interpretation would be consistent with findings of Kitchell and Carpenter (1987) in Lake Michigan. Such relatively high export of phytoplankton-derived OM to the sediment have been contributing to microbial respiration and appearance of anoxia at the lake bottom during summer. Because of such anoxia, phytoplankton-derived OM should be better preserved in the sediment. In summary, top-down effects of the less abundant community of small-bodied cladocerans on the phytoplankton community could result in a relatively high export of phytoplankton-derived OM to the sediment that is still responsible for temporary summer anoxia at the lake bottom and higher preservation of OM and pigments, despite reoligotrophication (Fig. 6E). These

two interacting processes might explain why the decreasing primary production over the past 30 yr was not recorded by the paleolimnological proxies related to sediment OM (TOC,  $\delta^{13}\text{C}$ , pigments). Such preservation effects, however, might not have significantly affected the interpretation of changes in cladoceran remains as paleolimnological and historical monitoring data are consistent during the reoligotrophication period.

**Other perturbations**—The combination of paleoecological and stable isotope approaches could successfully document irreversible changes in Lake Annecy food web over the last 150 yr. Changes in nutrient concentrations, in interaction with changes in predation pressure on *Daphnia* sp. as a result of whitefish continuous stocking, were identified as major drivers of these long-term changes. These two perturbations were certainly not the only ones that Lake Annecy has faced over the last century, among which the introduction of the zebra mussel (*Dreissena polymorpha*) in 1928 or climate change are the most important ones. However, no trend in our data actually supports a substantial effect of zebra mussel on the planktonic food web. In addition, long-term monitoring of water temperature of nearby lakes (Anneville et al. 2002) shows no significant increase in lake water temperature before the late 1980s and, as shown by historical monitoring data, complete mixing occurs in winter. Hence, if these two additional perturbations might have contributed to affect the reconstructed lake trajectory, we believe their contributions are negligible up to now.

Although whitefish were already introduced to Lake Annecy before eutrophication, intense stocking from the mid 1930s to the late 1990s has considerably altered the lake planktonic food web and prevented lake recovery along a simple reciprocal pathway.

Changes at the primary producer levels in the lake were essentially bottom-up driven, although a top-down effect on the export of phytoplankton-derived OM to the sediment during reoligotrophication might be responsible for the maintenance of summer temporary anoxia at the lake bottom. Changes at the cladocera levels were both bottom-up and top-down driven, with abundance in food resource controlling *Daphnia* sp. abundance and whitefish predation triggering reduction in *Daphnia* sp. size indirectly responsible for major changes in *Bosmina* sp. community structure. Further studies should provide information on the factors causing the reduction in *Daphnia* sp. size to evaluate the relative contribution of changes in *Daphnia* sp. composition, phenotypic plasticity, and microevolution to such processes (Hairston and De Meester 2008). And last, this first attempt to perform SIA on subfossil remains revealed that these top-down-driven changes in cladoceran community structure came along with major changes in the food-web structure, with the *Bosmina* group shifting from a low to a higher trophic position. Low abundance of *Daphnia* remains in samples did not allow the inclusion of this group in this study, but future work will investigate whether reduction in *Daphnia* size contributed to lower their trophic position within the food web. This study illustrates that SIA of subfossil

remains is a promising approach to reconstruct past changes in lake food webs.

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